

Phytochemical and Antiulcerogenic Properties of Rhizomes from *Simaba ferruginea* St. Hill. (Simaroubaceae)

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Simaba ferruginea (Simaroubaceae) is a Brazilian medicinal plant used in traditional medicine to treat several ailments, including gastric ulcers, fever, diarrhea, and dolorous and inflammatory processes. This study examines the chemical composition and antiulcerogenic effects of rhizomes from this plant. Bioassay-guided fractionation led to the isolation of two bioactive indole alkaloids called canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**). The alkaloid fraction and both alkaloids demonstrated potent antiulcerogenic effects when evaluated in gastric lesion-induced animals, as well as significant antinociceptive activity in mice. These results confirm and justify the popular use of *S. ferruginea* against gastric ulcers and dolorous processes.

Key words: *Simaba ferruginea*, Antiulcerogenic Effect, Antinociceptive Effect, Alkaloids

Introduction

Simaba ferruginea St. Hill. (Simaroubaceae) is a Brazilian native plant which is well-distributed in the Cerrado and known as calunga or féo-da-terra. Its rhizomes are frequently used in folk medicine to treat gastric ulcers, fever, diarrhea, rheumatism, pain, and other disorders (Lorenzi and Matos, 2002; Marcello, 2001).

Although several plants belonging to the Simaroubaceae family are used in traditional medicine to treat various diseases, particularly malaria and digestive disorders, only a few chemical and pharmacological studies have been reported regarding the genus *Simaba* (Moretti *et al.*, 1994; Ozeki *et al.*, 1998; Toma *et al.*, 2002; Muhammad *et al.*, 2004).

Previous preliminary pharmacological studies carried out by our group with the alcoholic crude extract from the rhizomes of *S. ferruginea* demonstrated interesting antiulcerogenic effects against the ethanol and indomethacin gastric ulcer model (Marcello, 2001). These promising pharmacological results, and the absence of phytochemical investigations on this plant, encouraged us to eluci-

date its active principles by bioassay-guided fractionation, using experimental gastric lesions in laboratory animals. We also evaluated the possible analgesic potential of this plant on the writhing test in mice.

Material and Methods

Plant material

Rhizomes of *S. ferruginea* were collected in Cuiabá, in the State of Mato Grosso, Brazil, in October 2001. The material was identified by Dr. Germano Guarim Neto (Botanical Department, Universidade Federal de Mato Grosso) and authenticated by Ms. Harri Lorenzi (Instituto Plantarum de Estudos da Flora de São Paulo). A voucher was deposited at the Central Herbarium (Universidade Federal de Mato Grosso) under number 21.883.

Phytochemical analysis

2 kg of dried powdered rhizomes were macerated with methanol at room temperature for 7 d.

The extract was separated by filtration and concentrated under reduced pressure to obtain 78 g of a brown residue. A part of this material (35 g) was suspended with methanol and water (7:3) and successively partitioned with solvents of increasing polarity (Cechinel Filho and Yunes, 1998) to give the following fractions and yields: *n*-hexane (4.16 g), dichloromethane (DCM) (1.53 g) and ethyl acetate (EA) (0.6 g).

A part of the *n*-hexane fraction (2.2 g) was chromatographed using a silica gel column eluted with an *n*-hexane/acetone gradient. From this fraction, several compounds were detected, particularly fatty acids, steroids and alkaloids. The DCM fraction also demonstrated the presence of alkaloids, the same as those evidenced in the above case. For this reason, we prepared a specific alkaloid fraction, according to previously described methods (Ugaz, 1994). 15 g of the methanolic extract was used to obtain 1.51 g of alkaloid fraction. It was preliminarily analyzed by TLC and specific reagents, showing four spots, two in higher concentration. In view of the fact that of all the fractions pharmacologically evaluated, the alkaloid fraction was the one which showed the most pronounced activity, this was studied in more detail. Thus, 1 g of this fraction was chromatographed using a silica gel column eluted with *n*-hexane/acetone gradient, yielding two pure yellow amorphous powders, which were identified as canthin-6-one (**1**) (200 mg) and 4-methoxycanthin-6-one (**2**) (250 mg), on the basis of their spectral data (IR, ¹H and ¹³C NMR) compared with those published in the literature (Koike and Ohmoto, 1985; Facundo *et al.*, 2002).

Animals

Male albino Wistar rats (180–200 g) and male Swiss mice (30–35 g) were used. The animals were maintained in a temperature-controlled environment (26 ± 2 °C) with a 12 h light-dark cycle. Food and water were freely available during the acclimatization period of the animals. Groups of five to eight animals were used for experimentation.

Ethanol-induced gastric lesions

After fasting for 12 h, the mice were treated with the vehicle and samples [methanolic extract from rhizomes, fractions and isolated compounds canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**)] in a 5 or 20 mg/kg dose, administered orally (1 h) or via intraperitoneal injection (30 min) prior

to the administration of ethanol 50% (10 ml/kg). Thereafter, the animals were sacrificed by ether anesthesia. The stomachs were removed, opened along the greater curvature, then thoroughly rinsed with normal cold saline. The areas of gross hemorrhagic damage were traced on a glass slide and their sizes were estimated by projecting the tracing onto graph paper (Woo and Choo, 1994).

Gastric lesion induced by indomethacin

The method previously described by Djananguiri (1969) was used, with minor modifications. After fasting for 18 h with water *ad libitum*, the rats received the vehicle and treatment with the methanolic extract of rhizomes, fractions and compounds (dose 5 mg or 20 mg/kg) orally or intraperitoneally, according to the experiment. 1 h later, the ulcerogenic agent indomethacin, dissolved in 2% sodium bicarbonate solution, was administered orally to the rats at a dose of 30 mg/kg. After 3 h, the animals received the same vehicle and treatment again. The rats were sacrificed 6 h after the administration of an injurious agent with ether anesthesia. The stomachs were removed, opened along the greater curvature, then thoroughly rinsed with normal cold saline. The gastric lesion was quantified for attribution of the ulcer index (Robert *et al.*, 1979).

Abdominal constriction response caused by intraperitoneal injection of diluted acetic acid

Abdominal constriction was induced by intraperitoneal injection of 0.1 ml/10 g body weight of acetic acid (0.6%) according to the procedure described previously (Collier *et al.*, 1968; Souza *et al.*, 2003). The mice were pre-treated intraperitoneally (5 mg or 20 mg/kg) 30 min prior to the administration of acetic acid with a methanolic extract from rhizomes, fractions and compounds **1** and **2**. After the challenge, the mice were housed in individual cages and the number of abdominal constrictions was cumulatively counted over a period of 30 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions between the control animals and mice pre-treated with the tested material.

Statistical analysis

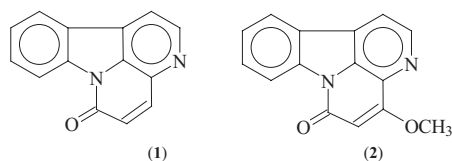
The results are presented as mean ± s.e.m. The parametric data were analyzed by one-way analysis of variance, and the statistical significance

among groups was determined by the Tukey-Kramer test. The ulcer index was analyzed by the non-parametric Kruskal-Wallis test, followed by the Dunnet's test. *P* values less than 0.05 were considered as indicative of significance.

Results and Discussion

In order to obtain the active principles of *S. ferruginea* rhizomes, we carried out a bioassay-guided fractionation. For this, we initially prepared a methanolic extract, which was partitioned into three fractions: *n*-hexane, dichloromethane and ethyl acetate. The first two fractions exhibited good antiulcerogenic activity and showed a strong presence of alkaloids. For this reason, a specific methodology was used to isolate these compounds. The alkaloid fraction yielded, by column chromatography, two indole alkaloids, named canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**) (Scheme 1).

Different kinds of biological activities have been reported for both compounds, such as antifungal (He *et al.*, 2002; Thouvenel *et al.*, 2003), leishmanicidal (Ferreira *et al.*, 2002), antileukemic (Fukamiya *et al.*, 1986), antimalarial (Kuo *et al.*, 2003), antiplasmodial (Chan *et al.*, 2004), anticancer (Ari-



Scheme 1. Canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**).

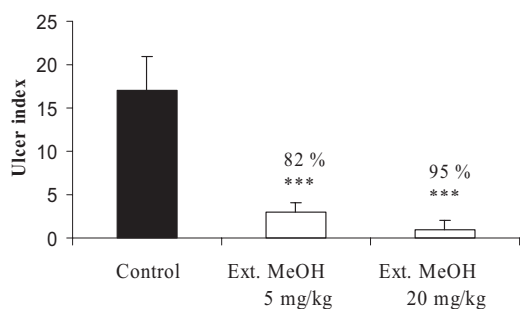


Fig. 1. Antiulcerogenic activity of methanolic extract (5 mg and 20 mg/kg intraperitoneal dose) against ethanol-induced gastric ulcers in mice. Each column represents the mean $\pm$ s.e.m. of five to eight experimental values. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

sawa *et al.*, 1983), and cytotoxic activity against human cancer cells (in the lung, breast, colon, fibrosarcoma, melanoma, KB, and others) (Anderson *et al.*, 1983; Kardono *et al.*, 1991; Kuo *et al.*, 2003). However, this is the first report concerning the presence of these alkaloids in *S. ferruginea*.

The methanolic extract from rhizomes (5 mg and 20 mg/kg i.p.) exhibited effective antiulcerogenic action, inhibiting the ulcer index in mice by 82% and 95%, respectively (Fig. 1), when analyzed in the ethanol-induced gastric ulcer model. However, the *n*-hexane, DCM and EA fractions were less active, suggesting a synergic effect or division of the same components in the fractions, like that observed with the alkaloids. On the other hand, the alkaloid fraction (5 mg and 20 mg/kg p.o.) inhibited 66% and 80% of index ulceration

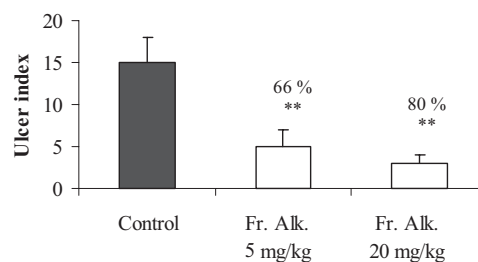


Fig. 2. Antiulcerogenic activity of alkaloid fraction (5 mg and 20 mg/kg oral dose) against ethanol-induced gastric ulcers in mice. Each column represents the mean $\pm$ s.e.m. of five to eight experimental values. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

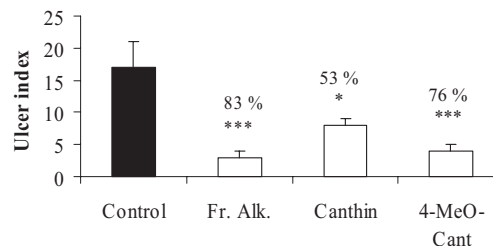


Fig. 3. Antiulcerogenic activity of alkaloid fraction and canthin-6-one and 4-methoxycanthin-6-one (20 mg/kg intraperitoneal dose) against ethanol-induced gastric ulcers in mice. Each column represents the mean $\pm$ s.e.m. of five to eight experimental values. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.

in mice, respectively (Fig. 2). These results are important, since the previously determined lethal dose (1800 mg/kg v.o. extract) is smaller than the beneficial dose (Marcello, 2001).

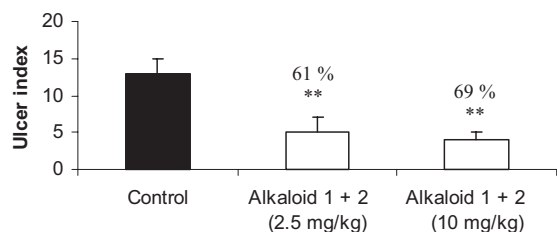


Fig. 4. Antiulcerogenic activity of a mixture of alkaloids, canthin-6-one and 4-methoxycanthin-6-one (2.5 mg/kg and 10 mg/kg each intraperitoneal dose) against ethanol-induced gastric ulcers in mice. Each column represents the mean $\pm$ s.e.m. of five to eight experimental values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

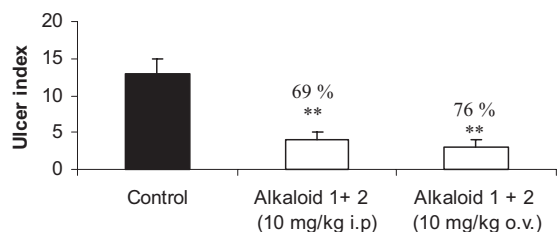


Fig. 5. Antiulcerogenic activity of a mixture of alkaloids, canthin-6-one and 4-methoxycanthin-6-one (10 mg/kg each intraperitoneal dose and oral dose) against ethanol-induced gastric ulcers in mice. Each column represents the mean $\pm$ s.e.m. of five to eight experimental values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**), predominant compounds of the alkaloid fraction, were tested in the same manner and also administered intraperitoneally. The results demonstrate a probable synergism between the alkaloids present in the fraction analyzed, since they were effective only in the 20 mg/kg dose (Fig. 3). The alkaloid fraction was effective in both doses (5 mg and 20 mg/kg i.p.) inhibiting 76% and 83% of the ulcer index, respectively.

In another experiment, the mixture of the two identified alkaloids (2.5 mg/kg and 10 mg/kg for each dose) was evaluated by the intraperitoneal

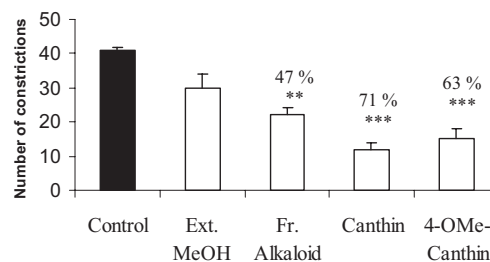


Fig. 6. Antinociceptive activities of methanolic extract, alkaloid fraction and canthin-6-one and 4-methoxycanthin-6-one (20 mg/kg intraperitoneal dose) against acetic acid-induced abdominal constrictions in mice. Each column represents the mean $\pm$ s.e.m. of six to eight experimental values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

route, inhibiting the ulcer index by 61% and 69%, respectively (Fig. 4). These results clearly demonstrate the existence of a possible synergic action between the two alkaloids, but the effect observed in the alkaloid fraction, which inhibited about 75%, suggests the presence of other active components in this fraction.

When administered orally, the mixture of alkaloids, at 10 mg/kg for each compound, inhibited 76% (Fig. 5). These results suggest that the alkaloid fraction and compounds **1** and **2** exhibit a significant dose-dependent reduction in ethanol-induced ulceration, indicating an antiulcerogenic action, since ethanol is well-known to promote oxygen-free radicals, reduce gastric mucus and stimulate the formation of leukotriene, to induce the formation of the gastric ulcer. Although there is controversy concerning the role of mucus in the prevention of gastric mucus injury, it acts as a considerable barrier for the prevention of gastric lesions (Sartori *et al.*, 1999; Repetto and Llesuy, 2002; Batista *et al.*, 2004).

On the other hand, the rats treated with the methanolic extract, fractions and compounds, by the intraperitoneal route, at doses of 5 mg/kg and 20 mg/kg, did not present significant results in the model indomethacin-induced gastric lesion, while

Treatment	Oral dose [mg/kg]	Median ^a	Q1–Q3	Significant
Vehicle	–	20	9–23	–
Methanolic extract	5	10	10–15	ns ^b
Methanolic extract	20	7	02–22	ns
Alkaloid fraction	5	4	2–7	*
Alkaloid fraction	20	1	0–2	**

Table I. Effect of methanolic extract and alkaloid fraction (5 mg and 20 mg/kg oral dose) in rats on gastric ulceration induced by indomethacin.

^a $n = 5–8$ in each group.

^b ns, not significant.

P (vs. control): * < 0.05 , ** < 0.01 .

for treatment by the oral route, at the same doses, only the alkaloid fraction demonstrated significant results in the inhibition of ulceration (Table I).

The analysis of rhizomes demonstrated that this plant is effective in the treatment of gastric ulcers, confirming its ethnopharmacological recommendation, since the antiulcerogenic effects of fraction and compounds observed are probably related to the production of gastric mucus and the inhibition of free radicals induced by ethanol. The alkaloid fraction and the compounds canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**) demonstrated promising antinociceptive activity against the writhing

test in mice at 20 mg/kg, administered intraperitoneally (Fig. 6). Compounds **1** and **2** inhibited around 71% and 63% of the abdominal constrictions, respectively, while the alkaloid fraction inhibited 47%. These results suggest a different profile for antiulcerogenic effect, in which the alkaloid fraction was more effective than the isolated alkaloids.

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